

1     1.     A method of identifying a therapeutic target, the method comprising the steps of:

2             (a) measuring protein or RNA levels of at least one component of an isolated mRNA  
3     ribonucleoprotein (mRNP) complex in a first sample enriched for a cell comprising a first  
4     phenotype; and

5             (b) comparing the levels determined in step (a) to the levels of the protein or RNA levels  
6     of the component in a second sample enriched for a cell comprising a second phenotype,

7             wherein if the levels of the component in the first sample are different from the levels of  
8     the component in the second sample, the component, a nucleic acid that encodes the component,  
9     or a protein encoded by the component is a potential therapeutic target for the treatment of a  
10    disease.

1     2.     The method of claim 1, wherein the cell comprising the first phenotype is selected from  
2     the group consisting of a mature adipocyte, a preadipocyte, pancreatic beta cell, a hepatocyte, a  
3     skeletal muscle cell, and a cardiac muscle cell.

1     3.     The method of claim 1, wherein the cell comprising the first phenotype is a mature  
2     adipocyte and the cell comprising the second phenotype is a preadipocyte.

1     4.     The method of claim 1, wherein the first phenotype is a disease related to glucose or lipid  
2     metabolism and the second phenotype is a normal phenotype.

1     5.     The method of claim 1, wherein the first phenotype is selected from the group consisting  
2     of obesity, diabetes, hypoglycemia, glucotoxicity, lipidtoxicity, insulin-resistance,  
3     hyperlipidemia, and lipodystrophy.

1     6.     The method of claim 1, wherein the component is selected from the group consisting of  
2     an RNA binding protein, an RNA, and an mRNP-associated protein.

1     7.     The method of claim 1, the method further comprising the step of:

2             (c) treating the sample in step (a) with an agent prior to measuring the protein or RNA  
3     levels of the component, wherein the agent alters the levels of at least one component of a  
4     glucose metabolic or a lipid metabolic pathway.

1 8. The method of claim 7, wherein the agent is selected from the group consisting of insulin,  
2 glucose, insulin-like growth factor-1 (IGF-1), a  $\beta$ -adrenergic agonist, glucose, glucagon-like  
3 peptide-1 (GLP-1), fatty acid, a peroxisome proliferator activated receptor (PPAR) ligand, and  
4 insulin-like growth factor 2 (IGF-2).

1 9. The method of claim 7, wherein the agent is a test therapeutic.

1 10. The method of claim 7, wherein the agent is selected from the group consisting of a  
2 nucleic acid, a protein, a peptide, or a small molecule.

1 11. The method of claim 1 or 7, further comprising the step of isolating the component, a  
2 nucleic acid encoding the component, or a protein encoded by the component.

1 12. The method of claim 1, wherein the component is Polypyrimidine Tract Binding Protein.

1 13. The method of claim 1, wherein the RNA binding protein is selected from the group  
2 consisting of the RNA binding proteins identified in Figure 10 to Figure 22.

1 14. The method of claim 1, wherein the component comprises a tag.

1 15. The method of claim 1, wherein the component is an mRNA that encodes a protein  
2 selected from the group consisting of a kinase, a transporter, a phosphatase, channel protein, a  
3 protease, a receptor, a transcription factor, and a transferase.

1 16. The method of claim 1, wherein the component is selected from the group consisting of  
2 3-phosphoinositide dependent protein kinase-1, nuclear ubiquitous casein kinase 2, neural  
3 receptor protein-tyrosine kinase, MAP-kinase activating death domain, AMP-activated protein  
4 kinase beta-2 regulatory subunit, calcium/calmodulin-dependent protein kinase IV, Protein  
5 kinase C beta, adenylate kinase 3, mitogen activated protein kinase kinase 5, 6-phosphofructo-2-  
6 kinase/fructose-2,6-bisphosphatase 2, phosphatidylinositol 4-kinase, Glucokinase, glycogen  
7 synthase kinase 3 beta, phosphorylase kinase (gamma 2, testis), protein tyrosine phosphatase  
8 (non-receptor type 1), protein tyrosine phosphatase (non-receptor type 5), inositol  
9 polyphosphate-5-phosphatase D, Protein tyrosine phosphatase (receptor-type, zeta polypeptide),  
10 dual specificity phosphatase 6, protein tyrosine phosphatase (non-receptor type 12), glucose-6-  
11 phosphatase (catalytic), 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2, proton gated  
12 cation channel DRASIC, Sodium channel (nonvoltage-gated 1, alpha (epithelial)), calcium

13 channel (voltage-dependent, alpha2/delta subunit 1), Potassium inwardly-rectifying (channel,  
14 subfamily J, member 6), potassium channel regulator 1, calcium channel (voltage-dependent, T  
15 type, alpha 1G subunit), cyclic nucleotide-gated cation channel, amiloride-sensitive cation  
16 channel 1, potassium inwardly-rectifying channel J14, potassium large conductance calcium-  
17 activated channel (subfamily M, alpha member 1), potassium voltage gated channel (Shab-  
18 related subfamily, member 2), potassium channel subunit (Slack), potassium intermediate/small  
19 conductance calcium-activated channel (subfamily N, member 1), Sodium channel (voltage-  
20 gated, type V, alpha polypeptide), amiloride-sensitive cation channel 2 (neuronal), potassium  
21 channel (subfamily K, member 6 (TWIK-2)), cation-chloride cotransporter 6, solute carrier  
22 family 21 (organic anion transporter, member 12), amino acid transporter system A2,  
23 peptide/histidine transporter, choline transporter, solute carrier family 31 (copper transporters,  
24 member 1), solute carrier family 13 (sodium-dependent dicarboxylate transporter), solute carrier  
25 family 2 (facilitated glucose transporter, member 13), solute carrier family 12 (potassium-  
26 chloride transporter, member 5), Solute carrier family 6 (neurotransmitter transporter, serotonin,  
27 member 4), Solute carrier family 2 A2 (glucose transporter, type 2), carboxypeptidase D,  
28 ubiquitin specific protease 2, mast cell protease 1, proprotein convertase subtilisin / kexin, type  
29 7, laminin receptor 1 (67kD, ribosomal protein SA), protein tyrosine phosphatase (non-receptor  
30 type 1), calcium-sensing receptor, neural receptor protein-tyrosine kinase, glutamate receptor  
31 (metabotropic 4), nuclear receptor subfamily 4 (group A, member 2), Neuropeptide Y5 receptor,  
32 protein tyrosine phosphatase (non-receptor type 5), insulin-like growth factor 1 receptor, Protein  
33 tyrosine phosphatase (receptor-type, zeta polypeptide), nuclear receptor subfamily 4 (group A,  
34 member 3), glutamate receptor (metabotropic 1), Tumor necrosis factor receptor superfamily  
35 (member 1a), insulin receptor, gamma-aminobutyric acid receptor associated protein, protein  
36 tyrosine phosphatase, non-receptor type 12, cholinergic receptor (nicotinic, beta polypeptide 1),  
37 olfactory receptor (U131), Gamma-aminobutyric acid receptor beta 2, glial cell line derived  
38 neurotrophic factor family receptor alpha 1, Glycine receptor beta, glutamate receptor interacting  
39 protein 2, adenylate cyclase activating polypeptide 1 receptor 1, asialoglycoprotein receptor 2,  
40 adenosine A3 receptor, Fibroblast growth factor receptor 1, nuclear receptor binding factor 2,  
41 purinergic receptor P2Y (G-protein coupled 1), nuclear receptor subfamily 1 (group H, member  
42 4), peroxisome proliferator activator receptor(gamma), 5 hydroxytryptamine (serotonin) receptor  
43 4, retinoid X receptor gamma, insulin receptor-related receptor, putative N-acetyltransferase  
44 Camello 4, lecithin-retinol acyltransferase, Phenylethanolamine N-methyltransferase,  
45 fucosyltransferase 2, Sialyltransferase 8 (GT3 alpha 2,8-sialyltransferase) C, UDP-

46 glucuronosyltransferase, alpha 1,3-fucosyltransferase Fuc-T (similar to mouse Fut4),  
47 diacylglycerol O-acyltransferase 1, signal transducer and activator of transcription 3, ISL1  
48 transcription factor (LIM/homeodomain), and oligodendrocyte transcription factor 1.

1 17. The method of claim 16, wherein the protein is encoded by a gene selected from the  
2 group consisting of CNCG, CACNA2D1, KCNC3, and KCNB2.

1 18. A method for identifying a therapeutic target for the treatment of aberrant glucose  
2 metabolism or lipid metabolism, the method comprising the steps of:

3 (a) measuring RNA or protein levels of at least one component of an isolated mRNP  
4 complex in a first cell sample; and

5 (b) comparing RNA or protein levels determined in step (a) to the RNA or protein levels  
6 of the component from a second cell sample,

7 wherein if the levels of the component in the first sample are different from the levels of the  
8 component in the second sample, the component, a nucleic acid that encodes the component, or a  
9 protein encoded by the component is a potential therapeutic target for the treatment of the  
10 disease.

1 19. The method of claim 18, wherein the first cell sample is from an individual at risk of  
2 having a disease or who has a disease and the second cell sample is from a normal or healthy  
3 individual.

1 20. A method for identifying a therapeutic target related to the treatment of a disease, the  
2 method comprising the steps of:

3 (a) measuring RNA or protein levels of at least one component of an isolated mRNP  
4 complex in a sample that has been treated with an agent that alters the expression of a component  
5 of a glucose metabolic or lipid metabolic pathway; and

6 (b) comparing RNA or protein levels determined in step (a) to the RNA or protein levels  
7 of the component in an untreated control sample,

8 wherein if the levels of the component in the first sample are different from the levels of the  
9 component in the second sample, the component, a nucleic acid that encodes the component, or a

10 protein encoded by the component is a potential therapeutic target for the treatment of the  
11 disease.

1 21. A method for identifying a gene or gene product involved in a physiological pathway in a  
2 cell, the method comprising the steps of:

3 a. isolating an mRNP complex comprising at least one component that participates  
4 in a physiological pathway;

5 b. identifying at least one additional component of the isolated mRNP complex,  
6 wherein the additional component is also involved in a physiological pathway.

1 22. The method of claim 21, wherein the physiological pathway comprises a metabolic  
2 pathway or a regulatory pathway.

1 23. The method of claim 21, further comprising the step of confirming the activity of the  
2 additional component by inhibiting the expression of the additional component in a cell and  
3 determining the effect of the inhibition on metabolism.

1 24. The method of claim 23, wherein the inhibition step comprises inhibiting gene expression  
2 of the additional component using an agent selected from the group consisting of an RNAi, an  
3 antisense RNA, a ribozyme, and a PNA.

1 25. A method for identifying an agent that alters a physiological pathway, the method  
2 comprising the steps of:

3 a. subjecting a cell sample to an agent;

4 b. isolating an mRNP complex comprising at least one component that participates  
5 in a physiological pathway from the sample;

6 c. measuring the RNA or protein levels of at least one component of the isolated  
7 mRNP complex,

8 d. comparing the RNA or protein levels of step (c) to the RNA or protein levels of  
9 the component isolated from an untreated control sample,



10 wherein differential expression of the component in the agent-treated sample compared to the  
11 untreated control sample is indicative that the agent regulates the physiological pathway.

1 26. The method of claim 25, wherein the agent interacts with or regulates a component of the  
2 physiological pathway.

1 27. The method of claim 25, wherein the agent inhibits a physiological pathway.

1 28. The method of claim 25, wherein the agent enhances a physiological pathway.

1 29. The method of claim 25, wherein the physiological pathway is an insulin production  
2 pathway or a lipogenesis pathway.

1 30. A method for identifying a protein that regulates glucose metabolism, the method  
2 comprising the steps of:

3 a. measuring the expression in an isolated mRNP complex of at least one gene  
4 product of a cell involved in glucose metabolism, wherein the gene product is selected from the  
5 group consisting of an RNA binding protein, an mRNA associated with said RNA binding  
6 protein, or an mRNP complex-associated protein;

7 b. treating the cell with an agent selected from the group consisting of insulin,  
8 glucose, insulin-like growth factor-1 (IGF-1), a  $\beta$ -adrenergic agonist, glucose, glucagon-like  
9 peptide-1 (GLP-1), fatty acid, a peroxisome proliferator activated receptor (PPAR) ligand, and  
10 insulin-like growth factor 2 (IGF-2); and

11 c. measuring the expression of the gene product after treatment, wherein a  
12 difference in expression of the gene product after treatment compared to expression of the gene  
13 product before treatment is indicative that the protein regulates glucose metabolism.

1 31. A method for identifying an agent that regulates insulin production, the method  
2 comprising the steps of:

3 a. contacting a cell involved in insulin production with a nucleic acid capable of  
4 binding to at least one protein, wherein the protein is capable of binding to a 3' untranslated  
5 region or a 5' untranslated region of a preproinsulin mRNA;

- 6           b.       separating the nucleic acid from the protein; and
- 7           c.       identifying the protein.

1   32.     The method of claim 31, wherein the protein binds to a nucleic acid comprising a  
2   sequence selected from the group consisting of 5'-gaauaaaaccuuugaaagagcacuac-3', 5'-  
3   cccaccacuaccuguccaccccucugcaaug-3', and 5'-  
4   agccctaagtgaccagctacagtcggaaaccatcagcaagcaggtcattgtccaac-3'.

1   33.     An mRNP complex-associated with at least one of glucose or lipid metabolism, wherein  
2   the mRNP complex comprises a polypyrimidine tract binding (PTB) protein, and at least one  
3   mRNA associated with the polypyrimidine tract binding protein.

1   34.     A method for identifying a component of an mRNP complex, the method comprising the  
2   steps of:

- 3           (a) transfecting a cell sample with a nucleic acid that inhibits the expression of an RNA  
4   binding protein;
- 5           (b) isolating total RNA from the cell sample and from a control sample;
- 6           (c) identifying RNAs that have altered expression in the nucleic acid-transfected sample  
7   compared to the control sample.

1   35.     The method of any one of claims 1, 7, 18, and 20, wherein the disease is related to  
2   aberrant glucose or lipid metabolism.

1   36.     The method of claim 21 or 25, wherein the physiological pathway comprises a glucose or  
2   lipid metabolic pathway.

1   37.     The method of any one of claims 1, 17, 20, 25, and 30, wherein at least one of said  
2   measuring and said comparing steps comprises the use of an array.